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IN-VIVO ANTI-INFLAMMATORY ACTIVITY OF THE LEAF EXTRACTS AND FRACTIONS OF OCIMUM SUAVE IN MICE MODEL

BIRHANETENSAY MASRESHA

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In-vivo anti-inflammatory activity of the leaf extracts and fractions of O*cimum suave* in mice model

A thesis submitted to the School of Graduate Studies, Addis Ababa University in partial fulfillment of the requirements for the Degree of Master of Science in Pharmacology in the Department of Pharmacology, School of Medicine

By

Birhanetensay Masresha (B.Pharm)

Under the direct supervision of Prof. Eyasu Makonnen, Department of Pharmacology, School of Medicine, Addis Ababa University and Dr. Asfaw Debella, Directorate of Traditional and Modern Medicine, Ethiopian Health and Nutrition Research Institute

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ABBREVIATIONS AND ACRONYMS

AA	Arachidonic acid
AMP	Adenosine monophosphate
BK	Bradykinin
COX	Cycloxigenase
Cys-LTs	Cysteinyl Leukotrienes
GM-CSF	Granulocyte-Monocyte Colony Stimulating Factor
HETEs	Hydroxyeicosatetraenoic acids
HRs	Histamine receptors
ICAM-1	Intracellular Cell Adhesion Molecule
IFN	Interferon
IL	Interleukin
LOX	Lipoxigenase
LT	Leukotrienes
LXs	Lipoxins
NO	Nitric oxide
NOS	Nitric oxide Synthase
NSADs	Non Steroidal Anti-inflammatory Drugs
PAF	Platelet Activating Factor
PDE_4	Phosphodiesterase E_4
PG	Prostaglandin
PLA_2	Phospholipase A_2
PMNs	Polymorphonuclear cells
TGF-	Transforming Growth Factor
TM	Traditional Medicine
TNF-~	Tumor Necrosis Factor
TXs	Thromboxanes
VCAM	Vascular Cell Adhesion Molecule
WHO	World Health Organization
5-HPETE	5-Hydroperoxyeicosatetranoic acid
5-HT	5-Hydroxytryptamine

ABSTRACT

Ocimum suave has been used in Ethiopian traditional medicine to relieve pain, fever, inflammation and possibly other diseases conditions. Although there are some reports on analgesic, antipyretic, antiulcer, antimicrobial and wound healing effects, no study has been made regarding their possible anti-inflammatory effect. The objective of the present study was, therefore, to investigate ethanol and aqueous extracts and fractions (butanol and water residue) of the aqueous extract of the plant material. The ethanol and aqueous extract were screened for their anti-inflammatory activities on carrageenan-induced mouse paw edema at three dose levels 400, 600 and 800mg/Kg. The butanol and water fractions of the aqueous extract were also investigated for their anti-inflammatory activities on carrageenan, histamine and serotonin-induced mouse paw edema at three dose levels 50, 100, 200mg/Kg. Normal saline and aspirin (200mg/Kg) were employed as negative and positive control groups, respectively. Both ethanol and aqueous extracts significantly decreased carrageenan-induced inflammation at all doses used. Greater paw edema inhibition was, however, observed by the aqueous extract than ethanol one at 600 and 800mg/Kg dose levels. Similarly, both butanol and water fractions also showed significant reduction of inflammation induced by carrageenan, histamine and serotonin in mice paw. Highest inhibition, however, was exhibited by the aqueous fraction at 100mg/Kg and 200mg/Kg dose levels. From the present findings, it can be concluded that the extracts and fractions of the plant material have got anti-inflammatory properties. Thus the results support the traditional use of this plant in inflammatory conditions. Further investigation, however, should be pursued to identify the exact mechanism (s), isolate the bioactive principle (s) responsible for the anti-inflammatory action and others.

1. INTRODUCTION

1.1. Inflammation

Inflammation is considered immunological defense mechanism that is elicited in response to mechanical injuries, burns, microbial infections, allergens and other noxious stimulus (Yoon and Baek, 2005). The inflammatory response is a highly regulated biological process that enables the immune system to efficiently remove the injurious stimuli and initiate the healing process. It also establishes memory that enables the host to mount a faster and more specific response upon a future encounter, which leads ultimately to the restoration of tissue structure and function (Kular et al., 2011; Stables and Gilroy, 2011).

The process of inflammation is necessary in healing wounds. However, uncontrolled and persistent inflammation contributes to the progression of many chronic pathological conditions, such as rheumatoid arthritis, atherosclerosis, psoriasis, inflammatory bowel disease, retinitis, multiple sclerosis etc. (Kular et al., 2011; Talwara et al., 2011). It may also be associated with increased risk of cancer (Coussens and Werb, 2002; Schwab and Serhan, 2006; Mantovani et al., 2008; Aggarwal and Gehlot, 2009).

Inflammation could either be acute or chronic. The acute inflammatory response involves a complex series of events, including dilatation of arterioles, venules, and capillaries with increased vascular permeability, exudation of fluids including plasma proteins, and leukocyte migration into the inflammatory sites (Cirino, 1998; Delves and Roitt, 2002; Calder, 2006). This response produce the cardinal signs of inflammation redness (rubor), heat (calor), swelling (tumor), pain (dolor) and loss of function (De las Heras and Hortelano, 2009). It is a short-term response that usually results in healing process (Weiss, 2008).

The acute phase begins with the production of soluble mediators by resident cells in the injured or infected tissue that promote the exudation of proteins and influx of granulocytes from the blood. The leukocytes, typically neutrophils, phagocytose and eliminate foreign microorganisms. This process is followed by an event accompanied by active anti-inflammatory and pro-resolution processes (Stables and Gilroy, 2011).

In the chronic phase, inflammation is characterized by the development of specific humoral and cellular immune responses to the pathogens present at the site of tissue injury (Feghali and Wright, 1997). It is a prolonged, dysregulated and maladaptive response that involves active inflammation, tissue destruction and attempts at tissue repair (Weiss, 2008; Leitch et al., 2009). This phase involves infiltration of mononuclear immune cells: monocytes, macrophages, lymphocytes, and plasma cells (De las Heras and Hortelano, 2009).

Inflammatory process involves synthesis and release of local inflammatory mediators, such as prostaglandins (PGs), leukotrienes (LTs) and platelet activating factor (PAF) induced by phospholipase A_2 (PLA_2), cyclo-oxigenase (COXs) and lipo-oxigenase (LOXs) (Omoigui, 2007; Venkatesha et al., 2011). Besides, other important inflammatory mediators including chemokines, cytokines, vasoactive amines, kinins, nitric oxide (NO) are also released during inflammatory process (Omoigui, 2007; De las Heras and Hortelano, 2009; Dinarello, 2010).

1.1.1. Prevalence of Inflammatory Diseases

Unregulated inflammation can lead to persistent tissue damage and leads to a variety of inflammatory disorders. These include: rheumatoid arthritis, osteoarthritis, inflammatory bowel disease, multiple sclerosis, psoriasis, asthma, atopic dermatitis etc. (Nathan, 2002; Ward and O'Neill, 2003).

Osteoarthritis and rheumatoid arthritis are the most prevalent causes of disability in western countries (Bertolini et al., 2001). Rheumatoid arthritis commonly results in joint destruction and significant impairment in the quality of life of the patients. Population-based studies showed that rheumatoid arthritis affects 0·5-1·0% of adults, with 5-50 per 100000 new cases annually in developed countries. This disease is 2 to 3 times more common in women than in men and can start at any age, but most commonly starts in middle adult life (Quan et al., 2008; Scott et al., 2010).

Prevalence of rheumatoid arthritis varies geographically. The disease is common in Northern Europe and North America compared with parts of the developing world, such as rural West Africa (Scott et al., 2010). Studies from south European countries also suggested a relative

lower occurrence of rheumatoid arthritis when compared with North European and North American countries (Alamanos et al., 2006). In general, the prevalence of the disease appears to be lower in developing countries (Mody, 2008).

Osteoarthritis is a progressive rheumatic diseases characterized by loss of joint cartilage that leads to pain and loss of function primarily in the knees and hips. It affects 9.6% of men and 18% of women aged more than 60 years. Increases in life expectancy and aging populations are expected to make osteoarthritis the fourth leading cause of disability by the year 2020 (Gautam and Jachak, 2009).

Asthma is a major public-health problem whose prevalence is increasing in most developed countries. Allergic diseases including asthma are reported to be low in rural subsistence and economically developing communities, and they increase with adoption of Western lifestyle (Yemaneberhan et al. 1997; Tattersfield et al., 2002).

Prevalence of wheeze and asthma has great geographical variation. The reported prevalence of wheezing illness in Western countries varies from as low as 5% in Sweden to as high as 25–30% in Australia and New Zealand. Data from developing countries also show wide variation: 0.007% in Papua New Guinea, 3.3% in rural Tanzania, 9.8% in urban Abidjan, 11.4% in Nairobi, and 20% in the Maldives (Hailu et al., 2003).

Prevalence data concerning asthma in Ethiopia is limited. Yemaneberhan *et al*. (1997) reported a 3.6% and 1.3% prevalence of asthma in urban and rural Jimma, respectively. All respiratory symptoms were rare in children and significantly less common in the rural community. Rosenberg *et al*. (1999) reported asthma rates of 2.5% among Ethiopian Jews at the time of immigration to Israel, with an increase to about 17% after 8–17 years in their new country.

Epidemiological data concerning skin inflammatory diseases in many rural sub-Saharan Africa is scarce. However, the limited data available indicates that inflammatory skin diseases represent the most frequent causes of morbidity in developing countries. The case is no different in Ethiopia where both hospital and population-based studies revealed that

inflammatory skin disorders are the leading causes of morbidity in the country with the prevalence estimates of about 50–70% (Yonathan et al., 2006).

1.1.2. Chemical Mediators of Inflammation

Different types of mediators are released during inflammatory processes. These mediators can be derived from plasma proteins or secreted by cells (Medzhitov, 2008). Inflammatory mediators that can be involved in inflammatory reaction include: histamine, 5-HT, kinins, complement components, fibrin degradation product, lipid mediators (eicosanoids and PAF), cytokines, chemokines, proteolytic enzymes and NO (White, 1999; Medzhitov, 2008).

Eicosanoids

Arachidonic acid (AA)-derived eicosanoids belong to a complex family of lipid mediators that regulate a wide variety of physiological responses and pathological processes. Although originally recognized for their capacity to elicit biological responses, such as vascular homeostasis, protection of the gastric mucosa and platelet aggregation, eicosanoids are now understood to regulate immunopathological processes ranging from inflammatory responses to chronic tissue remodeling, cancer, asthma, rheumatoid arthritis and autoimmune disorders (Harizi et al., 2008; Haeggström et al., 2010).

Eicosanoids, including PGs, TXs, LTs and LXs, are signalling molecules that are generated primarily through an oxidative pathway from AA (Serhan and Chiang, 2008; Huwiler and Pfeilschifter, 2009). Unlike histamine, eicosanoids are not preformed in cells but are generated on demand (Rang et al., 2003).

The biosynthesis of eicosanoids depends on the availability of free AA. When tissues are exposed to diverse physiological and pathological stimuli, such as growth factors, hormones or cytokines, AA is produced from membrane phospholipids by the action of PLA_2 enzymes and can then be converted into different eicosanoids. AA can be enzymatically metabolized by three main pathways: Cytochrome P450 epoxygenase, COXs and LOXs (Figure 1). The P450 epoxygenase pathway produces HETEs and epoxides. The COX-1 and COX-2 generate PGs

and TXs; LOXs produce LTs and lipoxins (LXs) (Rolin et al., 2006; Harizi et al., 2008; Medzhitov, 2008).

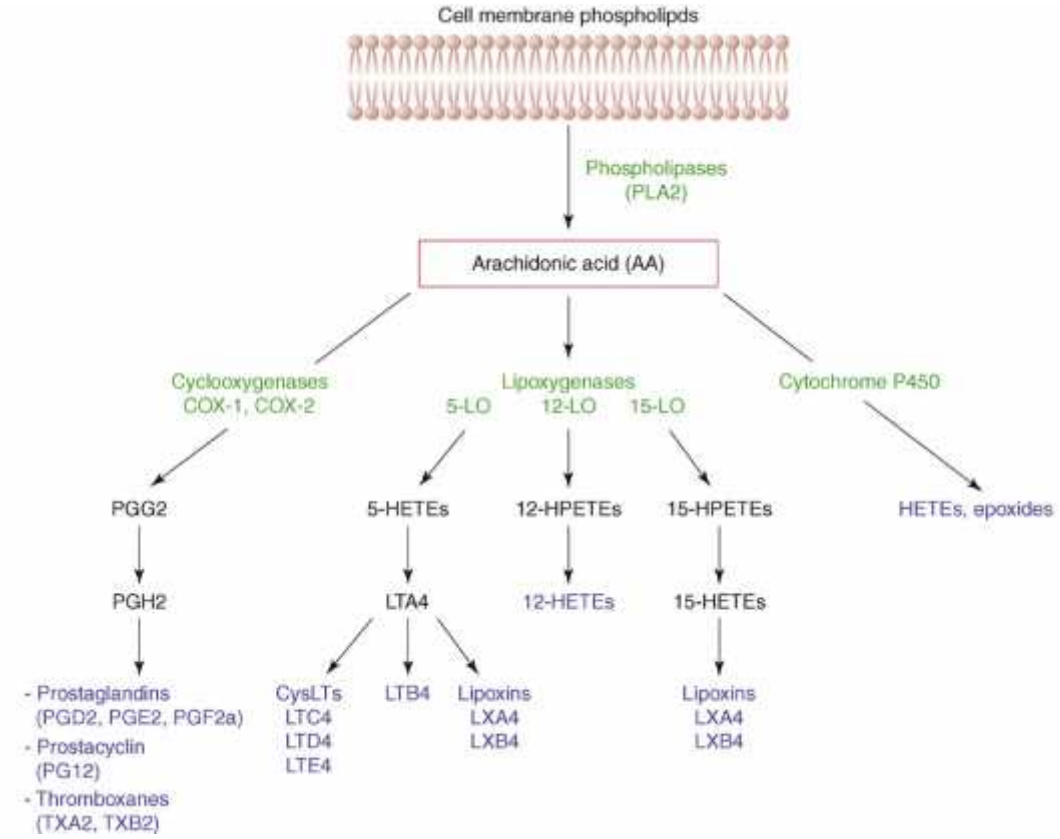

Fig.1: Eicosanoids biosynthesis from AA (Harizi et al., 2008).

Prostanoids are the end products of the metabolism of AA by COX, PG synthases, and TX synthase (Harris et al., 2002; Rolin et al., 2006; Huwiler and Pfeilschifter, 2009). These synthases include PGD synthase, PGE synthase, PGF synthase, PGI synthase, and TXA synthase, which form PGD_2, PGE_2, $PGF_{2\alpha}$, PGI_2 (prostacyclin) and TXA_2, respectively (Stables and Gilroy, 2011).

COX is a key enzyme in the biosynthesis of prostanoids from AA (Bertolini et al., 2001). In mammalian cells, COX exists in at least two isoforms: COX-1 (encoded by a constitutively expressed gene) and COX-2 (encoded by an immediate early response gene) (Harizi et al.,

2008; Stables and Gilroy, 2011), one variant form (COX-3) has been reported as well (Yoon and Baek, 2005).

COX-1 is constitutively expressed and responsible for basal production of prostanoids whereas COX-2 is inducible in response to different inflammatory stimuli such as endotoxin, IL-1β, and TNF-α (Hseu et al., 2005; Rolin et al., 2006). COX-1 is expressed in most mammalian cells under physiological conditions, and particularly large amounts are produced by endothelial cells, platelets, and kidney tubule cells. It has been shown that COX-2 is also constitutively expressed in the developing kidney and brain, playing a role in their maturation and function (Dannhardt and Kiefer, 2001; Ulbrich et al., 2002). Generally, COX-1 is involved in cellular housekeeping functions necessary for normal physiological activity whereas COX-2 participates in inflammation (Harris et al., 2002; Ulbrich et al., 2002).

Prostanoids are generated in most tissues and cells, modulating biological processes such as smooth muscle tone, vascular permeability, hyperalgesia, fever and platelet aggregation (Stables and Gilroy, 2011). PGE_2 and PGI_2 are the predominant pro-inflammatory prostanoids (Huwiler and Pfeilschifter, 2009). Both enhance vasodilation, oedema formation and vascular permeability particularly in the presence of histamine, bradykinin (BK) and 5-HT (Dannhardt and Kiefer, 2001; Rolin et al., 2006). PGE_2 is also the most potent pyretic agents and causes inflammatory pain (Dannhardt and Kiefer, 2001). Recent works demonstrated that PGI_2 also presents anti-inflammatory properties (Ley, 2008). In addition, PGD_2 is known to be associated with inflammatory conditions (Rolin et al., 2006).

Prostanoids exert their biological effects by binding to specific cell-surface receptors. There are at least nine known prostanoid receptors: the PGD receptors DP1 and DP2, the PGE_2 receptors, EP1, EP2, EP3 and EP4; the PGF receptor, FP; the PGI receptor, IP; and the TXA receptor, TP. All belong to the G-protein coupled receptor super-family (Rang et al., 2003).

LTs are end products of the metabolism of AA by 5-LOX. They are produced by various types of cells including mast cells, basophils, eosinophils, neutrophils, macrophages, monocytes (White, 1999; Kay, 2001). The first active intermediate derived from 5-LOX action is 5-HPETE which is further converted by synthases to the unstable LTA_4. Then LTA_4 may either

be converted to LTB_4 by a LTA_4 hydrolase, or may be conjugated with glutathione yielding LTC_4 by a LTC_4 synthase. Successive amino acid cleavage of LTC_4 yields LTD_4 and then LTE_4 (Rådmark and Samuelsson, 2010). LTC_4, LTD_4, and LTE_4 are collectively known as cysLTs because of the presence of a thioether-linked peptide (Fiorucci et al., 2001).

LTs act through four subtypes receptors, BLT-1, BLT-2, cysLT1, and cysLT2. They have physiological roles in innate immune responses and pathological roles in a variety of inflammatory and allergic diseases, such as rheumatoid arthritis, inflammatory bowel disease, allergic rhinitis, but most prominently in bronchial asthma. More recently, LTs have also been associated with atherosclerosis, osteoporosis and cancer (Huwiler and Pfeilschifter, 2009).

LTs are potent mediators of inflammation. For example LTB_4 causes: neutrophil, eosinophil, lymphocyte and monocyte chemotaxis; neutrophil and eosinophil aggregation; induction of neutrophil degranulation and lysosomal enzyme release; induction of neutrophil–endothelial cell adhesion; and modulation of pain induced by inflammatory reactions (Bertolini et al., 2001). Cys-LTs cause bronchial smooth muscle contraction and induce synthesis and release of other pro-inflammatory mediators such as IL-8, PAF, and known to have role in pathogenesis of asthma (Fiorucci et al., 2001; Rang et al., 2003).

There are other groups of lipid mediators, pro-resolving lipid mediators including LXs, resolvins, and protectins which are involved in inflammation (Serhan, 2008). LXs (LXA_4, LXB_4) are generated from AA by 15-LOX enzyme (Rang et al., 2003). They act to reduce inflammation and promote resolution. Resolvins and protectins are derived from omega-3 polyunsaturated fatty acid and they possess potent anti-inflammatory, neuroprotective and pro-resolving properties (Schwab and Serhan, 2006).

LXs were shown to exert their anti-inflammatory and pro-resolution effects through stopping infiltration and activation of PMNs, reducing the synthesis of the pro-inflammatory TNF-α and IL-8 and up-regulating the synthesis of anti-inflammatory TGF-β (Serhan, 2008; Huwiler and Pfeilschifter, 2009).

Platelet activating factor

PAF is an ether-linked phospholipid. It is generated by the acetylation of lysophosphatidic acid. PAF may be produced by several types of cells that participate in the inflammatory response including mast cells, macrophages, neutrophils, and eosinophils. It activates several processes that occur during the inflammatory response such as, recruitment of leukocytes, vasodilation, increased vascular permeability and platelet activation (White, 1999; Medzhitov, 2008).

Complement fragments

The complement system is a system of serine proteases which has role in host defense. Many complement products are pro-inflammatory. The complement fragments C3a, C4a and C5a are produced by several pathways of complement activation. C5a (and to a lesser extent C3a and C4a) promote granulocyte and monocyte recruitment and induce mast-cell degranulation (Gabay and Kushner, 1999; Ley, 2008).

Histamine

Histamine is a low molecular weight amine which is an important chemical mediator in physiological and pathological responses. The biological functions of histamine are mediated through G protein-coupled histamine receptors (HRs) including, H1R, H2R, H3R and H4R (Schneider et al., 2002; Zhang et al., 2007).

Histamine is a potent mediator of immediate hypersensitivity reactions and thus known to trigger allergic reactions such as anaphylaxis (Kay, 2001; Passani and Blandina, 2011). It is stored primarily in mast cells and basophils (Jadidi-Niaragh and Mirshafiey, 2010). Besides, histamine is also found in gastric enterochromaffin – like cells, and histaminergic nerves in the brain (MacGlashan, 2003). Histamine is a pro-inflammatory agent and has different effects in the body, causing increased vascular permeability and vasodilation, neutrophil and eosinophil chemotaxis and inflammatory mediator release (Jadidi-Niaragh and Mirshafiey, 2010).

Nitric oxide

Nitric oxide (NO) is a highly reactive free radical gas that is freely diffusible across membrane. It is synthesized and utilized by a variety of mammalian cells (Bishop and Anderson, 2005). NO is produced by the conversion of L-arginine to L-citrulline (Redington, 2006) via three different types of nitric oxide synthases (NOS) (Chatterjee and Catravas, 2008). Two of the isoforms endothelial (eNOS or NOS-3) and neuronal (nNOS or NOS-1) are expressed constitutively and responsible for the small amounts of NO production whereas the inducible isoform (iNOS or NOS-2) is expressed in response to a host of inflammatory stimuli such as bacterial products, cytokines, and lipid mediators to produce high amounts of NO (Redington, 2006; Blaise et al., 2007).

Studies showed that NO has role in the modulation of a variety of acute and chronic inflammatory disorders (Laroux et al., 2000). NO is generated at high levels during inflammatory reactions such as asthma and sepsis. The high and persistent level of NO exhibits cytotoxic action mediated by the production of reactive nitrogen species that lead to protein, cell and tissue damage (Coleman, 2001; Napoli et al., 2010). The overall effects of high NO are detrimental, including hypotension, pro-oxidant properties, apoptosis, and mediation of the effects of cytokines (Napoli et al., 2010).

Kinins

Kinins are potent peptide hormones formed de novo in body fluids and tissues during inflammation. They are derived from kininogens through proteolytic cleavage by kallikrein. Three types of kinins have been identified in humans: BK, kallidin and met-lys-BK. The actions of kinins are mediated by activation of two main BK receptor subtypes, the inducible (B1) and constitutive (B2) receptors (White, 1999; Blais Jr. et al., 2000; Abraham et al., 2006).

BK elicits responses that mimic all the cardinal signs of inflammation (redness, swelling, heat and pain) (Blais Jr. et al., 2000) related to B2 receptors. In chronic inflammatory processes, B1 receptor plays an important role (Costa-Neto et al., 2008). BK causes vasodilatation, increased vascular permeability and smooth muscle contraction. Its vasodilator action is partly as a result

of generation of PGI_2 and release of NO. It is a potent pain-producing agent, and its action is potentiated by PGs (Campbell, 2003; Rang et al., 2003).

Cytokines

Cytokines are low molecular weight intercellular signaling soluble proteins or polypeptides mediators synthesized and released by cells of the immune system during inflammation (Delves and Roitt, 2002; Verri Jr. et al., 2006). More than 100 cytokines have been identified, and includes interleukins, chemokines, interferons, colony-stimulating factors, growth factors and TNFs (Rang et al., 2003). They are produced by a variety of cell types, including macrophages, monocytes, mast cells, eosinophils and lymphocytes at inflammatory sites (Gabay and Kushner, 1999; Choy and Panayi, 2001).

Cytokines play roles in both acute and chronic inflammation exerting pro- or anti-inflammatory actions (Feghali and Wright, 1997; Kular et al., 2011). Among cytokines, IL-1, TNF-α, IFN-γ, IL-12, IL-18 and granulocyte-macrophage colony-stimulating factor are inflammatory, whereas IL-4, IL-10, IL-13, IFN-α and TGF-β are anti-inflammatory cytokines (Inagaki-Ohara et al., 2003).

Of the inflammatory cytokines, IL-1 (α and β) and TNF-α are extremely potent inflammatory molecules and are the primary mediators of septic shock (Feghali and Wright, 1997) and play pivotal role in the pathogenesis of rheumatoid arthritis (Lavagno et al., 2004). In addition, they are important cytokines in inducing the formation of other cytokines (Rang et al., 2003). For example: TNF-α induces IL-1, IL-6, IL-8, and GM-CSF (Choy and Panayi, 2001). Cytokines such as IL-1, IL-6, and TNF-α also induce PLA_2, COX-2 and iNOS enzymes (Cirino, 1998). Endothelial ICAM-1 and VCAM-1 expression is also increased by inflammatory cytokines, such as IL-1β or TNF-α (Ley, 2008).

Chemokines are also termed as chemotactic cytokines, which are released by leukocytes and other cells that regulate the transit of leukocytes from blood into tissues (Lewis and Manning, 1999; Delves and Roitt, 2002). Each type of leukocyte bears chemokine receptors that guide it to particular chemokines in the tissue (Delves and Roitt, 2002). In addition, chemokines also

have other cytokine-like activities, including regulation of angiogenesis, proliferation and apoptosis (Bonecchi et al., 2008). Chemokines are thought to provide the directional cues for the movement of leukocytes in development, homeostasis and inflammation (Luster, 1998).

1.1.3. Anti-inflammatory Drugs

Non Steroidal Anti-inflammatory Drugs

Non-steroidal anti-inflammatory drugs (NSAIDs) (such as, aspirin, ibuprofen, diclofenac) have been successfully used for the alleviation of pain, fever, and inflammation for many years and continue to be used daily by millions of patients worldwide (Rao et al., 2010; Vonkeman and van de Laar, 2010). NSAIDs are mainly indicated for mild to moderate pain and rheumatoid arthritis. Other indications include osteoarthritis, soft-tissue injury, renal colic, postoperative pain, and dental procedures (Vonkeman and van de Laar, 2010). The main mechanism of action of these drugs is believed to be the inhibition of the COX enzymes (COX-1 and COX-2) and consecutively the conversion of AA to PGs (Laine, 2002).

NSAIDs are well tolerated for short periods, but long-term administration may result in persistent adverse events (Quan et al., 2008). However, their use is limited by serious side effects, most common of which is gastroduodenal toxicity (Vonkeman and van de Laar, 2010). Other common adverse event is related to the kidneys i.e. acute renal insufficiency (Ziltener et al, 2010).

Since 1999, much attention has been directed to COX-2 selective inhibitors. COX-2 selective inhibitors (e.g. celecoxib, rofecoxib, valdecoxib, etoricoxib, lumircoxib) are class of NSAIDs that are used to treat rheumatoid and osteoarthritis, and other arthritic diseases, dental and surgical pain in post-operative settings, dysmenorrhoea, and acute injuries (Rainsford, 2007; Vonkeman and van de Laar, 2010). It has been observed that COX-2-selective NSAIDs could decrease the incidence of gastric and duodenal ulcers (Laine, 2002) by approximately 50 percent as compared with traditional NSAIDs (Quan et al., 2008). However, COX-2 inhibitors are associated with increased risk of cardiovascular events such as coronary thrombosis and stroke (Ward, 2008; Leitch et al., 2009).

Glucocorticoids

Glucocorticoids (corticosteroids or steroids) are the most effective anti-inflammatory agents available for many inflammatory and immune diseases including, asthma, rheumatoid arthritis, inflammatory bowel disease, and other autoimmune diseases (Barnes, 2006; Barnes and Adcock, 2009).

Glucocorticoids are able to bind with the cortisol receptors and trigger various biological effects (Quan et al., 2008). Although there are several mechanisms by which glucocorticoids reduce inflammation, a major one could be to reduce expression of cytokine-induced genes (Dinarello, 2010). Glucocorticoids inhibit the expression of pro-inflammatory cytokines such as IL-1, IL-2, IL-6, IL-8, TNF-α and IFN- γ. They also inhibit the generation of inflammatory mediators PGs, TXs, LTs and NO by suppression of gene expression of PLA_2, COX-2, iNOS (Pfeilschifter and Mühl, 1999). Further, they alter recruitment and activation of inflammatory cells such as, monocytes, macrophages, eosinophils or lymphocytes (Pfeilschifter and Mühl, 1999; Spies et al., 2010).

The adverse effects of glucocorticoids occur in different organs and the severity ranges from slightly cosmetic aspects to serious disabling and even life threatening situations. Systemic application of glucocorticoids usually causes more severe side effects than topical application. Some of the common side effects include osteoporosis, adrenal insufficiency, cataract, skin atrophy, peptic ulcers and risk of infection (Quan et al., 2008).

Anti-cytokine Drugs

Numerous cytokines play a critical role in many inflammatory diseases. Several strategies have been implemented to target these protein mediators such as the use of soluble monoclonal antibodies to bind the cytokines and/or receptor antagonists (Lewis and Manning, 1999).

TNF-α antagonists*:* TNF-α is a pleiotropic cytokine with both pro-inflammatory and immune-regulatory functions (Taylor, 2010). Thus, blockade of TNF-α has great importance in reduction of inflammation in different inflammatory diseases (Dinarello, 2010).

Currently there are three TNF-α inhibitors infliximab, etanercept and adalimumab that have been approved for treatment of different inflammatory diseases. All three of these TNF-α antagonist have shown significant and clinically relevant improvements in rheumatoid arthritis, Crohn's disease, moderate to severe psoriasis, ankylosing spondylitis and ulcerative colitis (Dinarello, 2010; Silva et al., 2010).

IL antagonists: IL-1 is a pro-inflammatory cytokine involved in arthritis. IL-6 is another pleiotropic inflammatory cytokine that has role in inflammation and hematopoiesis (Choy and Panayi, 2001). Two drugs, anakinra (IL-1 antagonist) and tocilizumab (IL-6 antagonist) are the current available agents (Dinarello, 2010) used for the treatment of rheumatoid and juvenile arthritis (Simmons, 2006).

Others

Antihistamines (H_1 antagonists) are drugs that are used to treat allergic rhinitis (and other allergic diseases). Commonly used antihistamines are loratadine, cetirizine, and fexofenadine as these drugs are less sedating and more selective H_1 blocker than earlier first generation antihistamines (Kay, 2001; Ward and O'Neill, 2003).

LT antagonists are another group of anti-inflammatory drugs which consist of the LOX inhibitors such as zileuton, and the cysLT receptor antagonists, such as montelukast and zafirlukast. These drugs are given to treat both rhinitis and asthma. They are effective in patients with mild to moderate asthma (Tattersfield et al., 2002; Rang et al., 2003).

Future Anti-inflammatory Agents

Neither the standard drugs nor the newer generations are without their shortcomings. Given the limitations of these agents, there remains a clear need for identification and validation of new anti-inflammatory drug targets (Ward, 2008). Some of the possible targets may include the following:

NO-NSAIDs: currently, the main focus of hybrid NO-NSAIDs development is to reduce the adverse effects observed with traditional NSAID (Quan et al., 2008). In the gastrointestinal

tract, NO is known to exert its protective role by increasing the mucous secretion, mucosal blood flow and inhibition of neutrophil aggregation (Rao et al., 2010). Clinical studies showed that NaproCINOD, an NO releasing derivative of naproxen, reduces adverse effects of NSAIDs including selective COX-2 inhibitors (Fiorucci, 2009).

PLA_2 inhibitors: PLA_2 is a key enzyme in the production of diverse mediators of inflammatory conditions (Quan et al., 2008). Inhibition of PLA_2 reduces production of oxidative products of AA (Buckle and Hedgecock, 1997). Several indole-based inhibitors of PLA_2 have been developed. Varespladib was developed as a treatment for rheumatoid arthritis and atherosclerosis and is under recent phase II trial (Rao et al., 2010). Another PLA_2 inhibitor LY333013 is in early phase clinical trial for treatment of arthritis (Quan et al., 2008).

Dual COX/5-LOX inhibitors: Drugs able to inhibit both COXs and 5-LOX have been designed in order to obtain compounds that retain the activity of classical NSAIDs while avoiding undesirable adverse effects. These compounds prevent pro-inflammatory and gastrointestinal damaging effects of LTs (Bertolini et al., 2001). ML3000 is the most potent and well balanced dual inhibitor of COX/5-LOX is now in clinical Phase III (Fiorucci et al., 2001).

PDE4 inhibitors: PDE4 is a major cyclic AMP-hydrolyzing enzyme in inflammatory and immunomodulatory cells. Inhibitors of PDE4 are anti-inflammatory and immunosuppressive agents. They cause inhibition of cellular trafficking and micro-vascular leakage, cytokine and chemokine release, reactive oxygen species production, and cell adhesion molecule expression (Sanz et al., 2005; Dinarello, 2010). These agents are currently under development for the treatment of asthma and chronic obstructive pulmonary disease (Spina, 2008). Rolipram, roflumilast, piclamilast, and pentoxifylline are in clinical use (Dinarello, 2010). Cilomilast is under phase II and III clinical trials. Others such as oglemilast and IPL512602 are currently in development (Spina, 2008). PDE4 inhibitors are also potential for the treatment of a number of other conditions such as allergic rhinitis, inflammatory bowel disease, atopic dermatitis, rheumatoid arthritis, and multiple sclerosis (Sanz et al., 2005).

Anti-adhesion agents: cell adhesion molecules (such as, integrins, selectins) are key mediators of inflammatory processes and are attractive targets for discovery of novel therapeutic agents

(Szekanecz and Koch, 2004; Simmons, 2005). These molecules are important to ingress leukocytes into sites of inflammation (Szekanecz and Koch, 2004). Different compounds are targeting these adhesion molecules. The monoclonal antibody efaluzimab, which binds the CD11a adhesion molecule, has been approved in some countries for the treatment of psoriasis. Natalizumab is anti-very late antigen-4 monoclonal antibody that showed great promise in the treatment of multiple sclerosis (Simmons, 2005; Simmons, 2006). Isis 2302 is other anti-adhesion compound which targets ICAM-1. There were positive data in Phase II trials for ulcerative colitis and these trials are continuing (Szekanecz and Koch, 2004; Simmons, 2005).

1.2. Medicinal Plants

The vast majority of people on this planet still rely on traditional medicine (TM) for their everyday health care needs (Gurib-Fakim, 2006). Plant medicines are the most popular traditional remedies (World Health Organization, 2008). It is known that many countries in Africa, Asia and Latin America rely on TM to meet some of their primary health care needs (World Health Organization, 2002). It is estimated that about 80% of the world's population primarily those of developing countries rely on plant-derived medicines for their healthcare needs (Gurib-Fakim, 2006). In many developed countries popular use of traditional/complementary and alternative medicine is also expanded due to great concern about the adverse effects of modern drugs (World Health Organization, 2002).

TM has been practiced in Ethiopia since long time ago. It is estimated that about 80% of the Ethiopian population is still dependent on TM, which essentially involves the use of medicinal plants (Giday et al., 2007). This might be due to the cultural acceptability of healers and local pharmacopeias, the relatively low cost of TM and difficult access to modern health facilities (Kassaye et al., 2006). The prevalence of the use of plant medicines in self-care was found to be 12.5% in rural central Ethiopia (Gedif and Hahn, 2003).

Despite an apparent lack of scientific evidence for the quality, safety and efficacy, African indigenous herbal medicines are widely used throughout the African continent (Salawu et al., 2008). Scientific evaluation and validation of traditional medicinal plants is, however, essential for their possible utilization or in elimination of harmful practices (Makonnen et al., 2003a).

Medicinal plants constitute a source of raw materials for both traditional and modern medicine. They are distributed worldwide, but they are most abundant in tropical countries (Gurib-Fakim, 2006). Medicinal plants are still indispensable source of medicaments in the contemporary health care delivery system (Abay, 2009). It is estimated that approximately one quarter of the best selling drugs worldwide were natural products or derived from natural products (Balunas and Kinghorn, 2005). In the case of certain classes of pharmaceuticals, such as anti-tumor and antimicrobial medicines, this percentage may be as high as 60% (Robinson and Zhang, 2011). Currently, 119 drugs of modern medicines are derived from 90 plants, of which 74% are from plants used in TM. Currently used modern drugs such as quinine, artmesnin derivatives, digoxin glycosides, tubocurarine, aspirin etc are good examples of drugs from plant medicine (Gurib-Fakim, 2006).

1.2.1. Plants with Anti-inflammatory Activity

The employment of anti-inflammatory agents may be helpful in the therapeutic treatment of those pathologies associated with inflammatory reactions (Sosa et al., 2002). Non-steroidal or steroidal anti-inflammatory drugs are commonly used to treat different inflammatory diseases (Yonathan et al., 2006; Boakye-Gyasi et al., 2008). However, the side effects of the currently available anti-inflammatory drugs pose a major problem in their clinical use (Tapiero et al., 2002; Yonathan et al., 2006). Attention is being given to the investigation of the efficacy of traditionally used plants as they are affordable and have fewer adverse effects (Adedapo et al., 2008; Saeed et al., 2010).

Since ancient times people have used phytochemicals found in plants to curtail the inflammatory process (Maroon et al., 2010). Some common plant medicines used to treat inflammation include: *Matricaria chamomilla* L. *Arnica montana* L., *Salix alba* (White Willow), *Glycyrrhiza glabra* (Shah et al., 2011), *Curcumin* (Turmeric), *pycnogenol* (maritime pine bark), *Boswellia serrata* resin (Frankincense), *Uncaria tomentosa (*Cat's Claw) (Maroon et al., 2010), *Cheilanthes farinose* (Yonathan et al., 2006), *Rosa abyssinica* Lindley, *Salvia nilotica* Juss (Sewuye and Asres, 2008), and *Clematis simensis* Fresen (Tadele et al., 2009).

The anti-inflammatory activities of plants are due to the secondary metabolites. These bioactive compounds consist of polyphenols, flavonoids, alkaloids, terpenoids, steroids, carotenoids, coumarins, curcumines etc (Saeed et al., 2010). Some of these secondary metabolites responsible for the anti-inflammatory activity are discussed briefly below.

Alkaloids

In recent years, a large number of different kinds of naturally occurring alkaloids with anti-inflammatory activity have been reported (Calixto et al., 2000; Marzouk et al., 2010). For example: berberine, berbamine, palmatine, oxyacanthine, magnoflorine, and columbamine isolated from the roots of Turkish *Berberis* species (Kupeli et al., 2002), fangchinoline and tetrandrine from *Stephania tetrandrae* roots (Gautam and Jachak, 2009), achyranthine from *Achyranthes aspera* L. (Singh et al., 2008), isoretuline from *Strychnos henningsii* Gilg (Calixto et al., 2000), pseudo-akuammigine from *Picralima nitida* (Duwiejua et al., 2003), and theacrine from *Camellia kucha* (Wang et al., 2010) showed anti-inflammatory activity in different models of inflammation.

Terpenoids

There are a number of plant derived terpenoids which exhibit distinctive pharmacological properties. Among other action, naturally occurring terpenoids have anti-inflammatory activity (Calixto et al., 2000). Some of them include: the sesquiterpene glucosides from methanol extract of *Taraxacum officinale* roots (Talhouk et al., 2007), triptolide from *Tripterygium wilfordii* (Talhouk et al., 2007; Gautam and Jachak, 2009), parthenolide from *Tanacetum parthenium* (Sobota et al., 2000), andrographolide from *Andrographis paniculata* (Singh et al, 2008), polygodial sesquiterpene from the bark of *Drymis winteri* (Da Cunna et al., 2001), and sesquiterpene lactone isolated from the methanolic extract of *Sphaeranthus indicus* (Fonseca et al., 2011).

Flavonoids

Flavonoids are polyphenolic compounds that occur ubiquitously in plants and have a variety of biological effects. They have been found to have antimicrobial, antiviral, anti-ulcerogenic, cytotoxic, anti-neoplastic, antioxidant, anti-hepatotoxic, antihypertensive, hypolipidemic, antiplatelet, anti-inflammatory and analgesic activities. Flavonoids also have biochemical effects, which inhibit a number of enzymes such as aldose reductase, xanthine oxidase, phosphodiesterase, Ca^{2+}-ATPase, PL, LOX, COX (Nijveldt et al., 2001; Rathee et al., 2009), and protein kinases (Manthey, 2000).

Different flavonoids with anti-inflammatory activity have been reported. The three flavonoids; 3-O-methylquercetin, 3,7-O-dimethylquercetin and 3,7-O-dimethylkaempferol from the ethanol extract of *Cistus laurifolius* L. (Küpeli and Yesilada, 2007); quercetin and dihydroquercetin from *Proustia pyrifolia* (Delporte et al., 2005); kaempferol-3,7-o-~ -dirhamnoside and quercetin-3,7-o-~ -dirhamnoside from leaves of *Tilia argentea* (Toker et al., 2004), baicalin from *Scutellaria baicalensis* Georgi (Li et al., 2000), nepetin, jaceosidin and hispidulin isolated from dichloromethane extract of *Eupatorium arnottianum* Griseb. (Clavin et al., 2007) exhibited anti-inflammatory effect.

1.3. Ocimum suave

One of the traditionally used plants believed to have anti-inflammatory component (s) is *Ocimum suave*. The genus *Ocimum*, a member of the Lamiaceae family, contains 200 species of herbs and shrubs. This species has a long history as culinary herbs, its foliage adding a distinctive flavor to many foods. It is also a source of aroma compounds and essential oils containing biologically active constituents (Hakkim et al., 2008).

Ocimum suave Willd is a plant belonging to the family Lamiaceae, syn.: Labiatae. The plant is found in tropical Asia and in West and East Africa where its geographical distribution is limited to mountainous areas (Tan et al., 2002). *Ocimum suave*, whose Oromifa name is “Anchabi”, is distributed in different regions of Ethiopia at a latitudinal range of 1600–2000 m above sea level (Makonnen et al., 2003b). *Ocimum suave* is one of the traditionally used plants

to relieve pain, fever and inflammatory disorders in Ethiopia. The mode of administration usually involves squeezing the fresh leaves and sniffing the juice, drinking or inhaling after boiling in water (Makonnen et al., 2003a). It is also used in traditional medicine for treatment of wounds in Uganda (Hassan et al., 2011) and for ulcers in East Africa (Tan et al., 2008). The Maandi, Koongo and Sundi peoples of Congo Brazzaville use the leaves of *Ocimum suave* in combination with other plants to treat fever in children, menstrual problems, stomach ache and broncho-pneumonic affections (Tan et al., 2008).

Phytochemical screening of both ethanol and aqueous extracts has revealed the presence of phenolic acids, flavonoids, glycosides and sugars as major chemical constituents (Makonnen et al., 2003b). Preliminary studies have also shown a high presence of triterpenes (Tan et al., 2008). Many volatile oil components either phenyl propane derivatives or terpenoids, including methyl eugenol, 1, 8-cineole, camphor, bornyl acetate, germacrene-D, E-myroxide, germacrene-B, caryophylene oxide and p-cymene have been identified (Runyoro et al., 2010).

Different scientific and pharmacological studies have been carried out on the plant *Ocimum suave*. Both the aqueous and ethanol leaves extracts of *Ocimum suave* showed analgesic activity using tail flick and hot plate (Makonnen et al., 2003a) as well as writhing method (Debella et al., 2003). Aqueous and ethanol leaves extracts of *Ocimum suave* also exhibited antipyretic activity in yeast induced fever (Makonnen et al., 2003b). Other studies showed that the leaves of *Ocimum suave* to possess gastric cytoprotective antiulcer effects on acute gastric ulcer (Tan et al., 2002) and healing effects on chronic acetic acid-induced gastric ulcers (Tan et al., 2005). Further studies also showed the mosquito repellent (Seyoum et al., 2002), antimicrobial (Runyoro et al., 2010) and wound healing activity (Hassan et al., 2011) of *Ocimum suave*.

Acute and sub chronic toxicity as well as teratogenic effects of the aqueous extract of the leaves of *Ocimum suave* were also evaluated in mice and rats. Following acute and sub chronic administration, the extract was well tolerated and neither produced overt signs of clinical toxicity nor any signs of hepato, nephro or haemato-toxicity. Teratogenic effects were also absent (Tan et al., 2008).

Though *Ocimum suave* is traditionally employed to relieve inflammatory disease conditions, it has not been confirmed scientifically. The present study was, therefore, carried out to justify the traditional use of the plant for inflammation.

2. OBJECTIVE

2.1. General Objective

The purpose of this study was to evaluate the anti-inflammatory activities of the crude extracts (ethanol and aqueous) and fractions (butanol and water residue) of the most active extract of *Ocimum suave* at different doses in mice.

2.2. Specific Objectives

- ✓ To evaluate the anti-inflammatory activity of aqueous and ethanol extracts of *Ocimum suave* using carrageenan inflammatory model.
- ✓ To evaluate the anti-inflammatory activity of the fractions (butanol and water residue) of the most active extract of *Ocimum suave* using carrageenan, histamine and serotonin inflammatory models.
- ✓ To get idea on the onset and duration of anti-inflammatory action of the plant

3. MATERIALS AND METHODS

3.1. Materials

3.1.1. Collection of Plant Materials

The leaves of *Ocimum suave* were collected from Jimma about 324 Km South West of Addis Ababa in January 2011. The plant was identified by a taxonomist and voucher specimens representing *Ocimum suave* (Herbarium No. 001) was deposited at the National Herbarium, Department of Biology, Addis Ababa University, Ethiopia.

3.1.2. Chemicals and Solvents

Carrageenan (Sigma chemical company, Lot 55H188), histamine dihydrochloride (Aldrich chemical company, batch No 16925PA), serotonin creatinine sulphate (BDH laboratory supplies), ethanol, n-butanol (India), chloroform (Research-Lab fine chemicals, batch No 30480601, Mumbai), acetylsalicylic acid (Zhong shanground, batch No.0605026, China) were employed for the experiment.

3.1.3. Experimental Animals

The experiments were performed on in-house bred albino male and female mice (weighing 20-30g), which were obtained from the Ethiopian Health and Nutrition Research Institute, Department of Pharmacology, School of Medicine, Addis Ababa University and Aklilu Lemma Institute of Pathobiology, Addis Ababa University. They were all acclimatized to the animal house prior to use. Mice were kept in cages in animal house with a 12 h light: 12 h dark cycle. They fed on pellets and tap water *ad libitum*. The care and handling of mice were in accordance with the internationally accepted standard guidelines for use of animals.

3.2. Methods

3.2.1. Preparation of Crude Extract

Three hundred grams of air dried and powdered leaves of *Ocimum suave* were macerated in 70% (V/V) ethanol for 72 hrs. After filtration, the extracts were then concentrated under vacuum in a rotary evaporator to give 8.53% (w/w) gummy residue. Four hundred and fifty grams of powdered leaves of the above plant were also separately macerated with cold water and lyophilized after filtration to give 10.15% (w/w) powdered residue. The extracts were kept in tightly stoppered bottles and stored in a dessicator for future use.

3.2.2. Preparation of Solvent Fractions

After anti-inflammatory screening test of the crude extracts, the most active extract i.e. aqueous extract was further fractionated using solvents: chloroform, butanol, and water. Fifty two grams of aqueous extract was taken and dissolved in warm distilled water. After filtration, the dissolved extract was added in a separatory funnel and mixed with 50 ml of chloroform three times. After shaking, it was allowed to stay for some time until complete formation of two layers. Then the lower layer i.e. chloroform was collected. After collecting the chloroform layer, 50 ml of butanol was successively added three times in the left aqueous residue and then the upper layer (butanol) was taken. Finally, the chloroform and butanol fractions were concentrated under vacuum in a rotary evaporator to give 0.67% (w/w) and 2.61% (w/w) gummy residue, respectively and the aqueous residue was lyophilized to give 17.31% (w/w) dried fraction. The fractions were kept in tightly stoppered bottles and stored in a dessicator till use. Based on the pilot experiment performed before commencing the actual experiments, butanol and water fractions were selected.

3.2.3. Testing Anti-inflammatory Activity

3.2.3.1. Carrageenan-induced Paw Edema

In vivo anti-inflammatory activity was evaluated on the basis of inhibition of carrageenan-induced mouse hind paw edema as previously described by Yonathan *et al*., (2006). Mice were

fasted for 12 hrs with free access to water before used for the experiments. Fourteen groups of mice of both sexes were used in this experiment and each group was composed of six mice (three male and three female). The first six groups received 400, 600 and 800mg/Kg of ethanol and aqueous extracts, while the other six groups were treated with 50, 100 and 200mg/Kg of butanol and water fractions. The remaining two groups received 200mg/Kg aspirin and normal saline that served as positive and negative controls, respectively. All the treatments were by oral gavage. A dose of 0.05 ml of 1% carrageenan in normal saline was injected into the sub-plantar surface of the left hind paw 30 minutes after the oral administration of the test substances, the standard and the vehicle. The volumes of injected paws were measured before, and 1, 2, 3, 4, 5 hrs after injection of carrageenan by plethysmographic method using Ugo-basile plethysmometer.

The increase in paw volume, i.e. inflammation was calculated according to the equation given by Delporte *et al*., (2005) and expressed in percentage:

$$\%I = \frac{V_f - V_i}{V_i} \times 100$$

Where, V_i is the volume of paw before carrageenan injection and V_f is the volume of paw after carrageenan injection at a given time.

In addition, the anti-inflammatory effect was calculated according to the formula given below Delporte *et al*., (2005) and expressed in percentage:

$$\%A = \frac{\%Ic - \%Ie}{\%Ic} \times 100$$

Where %Ic and %Ie are the mean inflammation values reached in control and experimental groups, respectively.

3.2.3.2. Histamine-induced Paw Edema

Mice were administered with the test substances (butanol and aqueous fractions), standard and vehicle as described in section 3.2.3.1. In this model edema of the left hind paw of the mouse

was induced by sub-plantar injection of 0.05 ml of 1% freshly prepared histamine as described by Gupta *et al*., (2006).

3.2.3.3. Serotonin-induced Paw Edema

Same procedure as described in sections 3.2.3.1 and 3.2.3.2 were followed except use of serotonin instead of carrageenan and histamine.

3.2.4. Statistical Analysis

The results were expressed as mean ± S.E.M. Statistical analysis of the data was analyzed using Students t - test. Results with $P<0.05$ were considered statistically significant.

4. RESULTS

4.1. Effects of *Ocimum suave* on Carrageenan-induced Paw Edema

The ethanol and aqueous extracts of the leaves of *Ocimum suave* reduced carrageenan-induced paw edema at all doses employed and both extracts showed dose-dependent anti-inflammatory effects (table 1). The smallest dose of the ethanol extract, 400mg/Kg, significantly reduced carrageenan-induced paw volume (*P<0.05*) at the 4 hour after administration. Ethanol extract at 600mg/Kg and 800mg/Kg, however, significantly reduced carrageenan-induced paw volume starting 1st hour after administration as compared to the control (*P<0.05*) (Table 1).

As shown in table 1, the aqueous extract reduced paw volume significantly at the dose of 400mg/Kg (*P<0.05*, and at the 3rd hour of administration, *P<0.01*). The result also showed that dose of 600mg/Kg significantly decreased paw edema (*P<0.05* at the 1st, 4th and 5th hours, and *P<0.01* at 2nd and 3rd hours). The highest dose of aqueous extract, 800mg/Kg, also produced significantly higher reduction in carrageenan-induced paw volume (*P<0.01* at the 1st hour and *P<0.001*at the rest of the hours) as compared to the control group (table 1).

Table 1: Effects of oral ethanol and aqueous extracts of *Ocimum suave* on carrageenan-induced mouse paw edema.

Group	Dose (mg/Kg)	Mean Inflammation (%I) ± S.E.M				
		1h	2h	3h	4h	5h
Normal saline		55.74 ± 4.31	71.33±4.86	72.90±4.06	68.36±5.32	62.41±5.53
Aspirin	200	33.49±4.01^{xb}	40.63±1.56^{xa}	45.79±2.07^{xa}	38.01±3.40^{xa}	32.03±3.33^{xa}
Ethanol extract	400	44.77±3.03^{yc}	57.42±4.62^{yb}	61.10±6.66	52.28±4.85$^{xc, yc}$	50.98±7.97^{yb}
	600	41.35±4.53^{xc}	53.53±2.98$^{xc, yb}$	55.36±5.30^{xc}	51.15±3.83$^{xc, yc}$	45.54±3.14$^{xc, yc}$
	800	40.87±2.00^{xc}	52.76±3.29$^{xc, yc}$	55.35±1.59$^{xb, yb}$	49.77±3.96$^{xc, yc}$	43.27±4.51^{xc}
Aqueous extract	400	40.67±3.79^{xc}	53.53±3.19$^{xc, yb}$	54.72±3.15$^{xb, yc}$	49.01±4.24^{xc}	43.41±5.07^{xc}
	600	39.44±4.52^{xc}	50.81±4.23^{xb}	54.72±3.15^{xb}	48.12±4.87^{xc}	40.60±5.86^{xc}
	800	33.72±3.02^{xb}	40.26±4.56^{xa}	43.99±4.23^{xa}	37.43±4.13^{xa}	30.66±3.86^{xa}

^{x}: relative to control, ^{y}: relative to standard: $^{a}P< 0.001$, $^{b}P< 0.01$, $^{c}P<0.05$.

The anti-inflammatory activities of ethanol and aqueous extract were shown in table 2. The ethanol and aqueous extract at a dose of 800 mg/kg oral exhibited maximum anti-inflammatory activity (30.67% and 50.87%, respectively) at the end of the 5th hour of administration (table 2). Although the ethanol extract produced lower effects than that of the standard drug, aspirin, high doses of aqueous extract were able to show comparable anti-inflammatory activities. The anti-inflammatory activity of aqueous extract at a dose of 800mg/Kg was a bit higher than aspirin (50.87% vs 48.68%) (Table 2). Greater paw edema inhibition was also observed by the aqueous extract than the ethanol one at 600 and 800mg/Kg dose levels.

Table 2: Anti-inflammatory activities of ethanol and aqueous extracts of *Ocimum suave* in comparison with that of aspirin on carrageenan-induced mouse paw edema.

Group	Dose (mg/Kg)	Anti-inflammatory activity (%A)				
		1h	2h	3h	4h	5h
Normal saline		—	—	—	—	—
Aspirin	200	39.91	43.04	37.19	44.39	48.68
Ethanol extract	400	19.68	19.51	16.18	23.52	18.31
	600	25.82	24.95	24.06	25.17	27.03
	800	26.68	26.03	24.07	27.19	30.67
Aqueous extract	400	27.04	24.95	24.94	28.31	30.44
	600	29.24	28.77	25.89	29.61	34.95
	800	39.50	43.56	39.66	45.24	50.87

Effects of the fractions of the aqueous extract on carrageenan-induced paw edema were shown in table 3 and 4. The butanol and water fractions reduced carrageenan-induced paw edema at all dose levels employed. As shown in table 3, dose dependent anti-inflammatory effects were observed for both fractions. The butanol fraction significantly decreased carrageenan-induced paw edema ($P<0.05$) at 4th and 5th hours at a dose of 50mg/Kg. At a dose of 100mg/Kg the butanol fraction also reduced paw volume significantly ($P<0.01$) at the 2nd and the 5th hours and ($P<0.001$) at the 3rd and the 4th hours). Significant reduction of edema was also observed

with 200mg/Kg butanol fraction at all time (*P<0.05* at 1st hour, *P<0.01* at 2nd and 5th hours, and *P<0.001* at 3rd and 4th hours) as compared to the control.

In similar manner, water residue at the smallest dose, 50mg/Kg, showed significant reduction of paw edema (*P<0.05* at 1st and 2nd hours, *P<0.001* at 3rd, 4th and 5th hours). It was also observed that 100mg/Kg and 200mg/Kg doses of water residue produced higher reduction of edema (*P<0.01* at 1st hour, and *P<0.001* at 2nd, 3rd, 4th and 5th hours) as compared to the control group.

It was also noted that the butanol fraction at 50 and 100mg/Kg produced significantly lower effect than aspirin on carrageenan-induced paw edema. However, the effect produced by 200mg/Kg butanol fraction and all doses of aqueous fraction were not significantly different from aspirin (Table 3).

Table 3: Effects of oral butanol and water fractions of *Ocimum suave* on carrageenan-induced mouse paw edema.

Group	Dose (mg/Kg)	Mean Inflammation (%I) ± S.E.M				
		1h	2h	3h	4h	5h
Normal saline		47.41±1.20	60.24±1.13	74.04±1.66	64.04±1.78	59.18±1.49
Aspirin	200	33.89±2.45^{xb}	37.70±2.70^{xa}	48.35±2.46^{xa}	37.67±2.97^{xa}	35.50±2.56^{xa}
Butanol fraction	50	43.25±1.90^{yc}	54.31±3.11^{yb}	63.84±4.78^{yc}	53.18±3.84$^{xc, yb}$	47.69±3.85$^{xc, yc}$
	100	42.76±2.24^{yc}	47.36±3.02$^{xb, yc}$	53.73±2.14^{xa}	45.81±1.64$^{xa, yc}$	42.36±3.32$^{xb, yc}$
	200	38.20±2.89^{xc}	42.47±3.54^{xb}	50.98±1.59^{xa}	44.42±1.58^{xa}	40.59±3.44^{xb}
Water residue	50	38.40±2.94^{xc}	47.73±3.74^{xc}	50.23±3.85^{xa}	46.89±2.48$^{xa, yc}$	40.42±2.37^{xa}
	100	35.03±3.01^{xb}	38.26±1.39^{xa}	46.87±2.32^{xa}	41.59±2.69^{xa}	35.86±1.43^{xa}
	200	32.13±3.57^{xb}	37.57±3.51^{xa}	45.24±2.41^{xa}	35.15±1.44^{xa}	31.56±1.78^{xa}

^{x}: relative to control, ^{y}: relative to standard: $^{a}P< 0.001$, $^{b}P< 0.01$, $^{c}P<0.05$.

Table 4 shows the anti-inflammatory activity of butanol and water fraction. The water fraction exhibited higher inhibition of paw edema than the butanol fraction at high doses. The butanol fraction showed 19.42% at the 5th hour with 50mg/Kg, 28.47% at the 4th hour with 100mg/Kg,

and 31.41% at the 5th hour with 200mg/Kg as their peak anti-inflammatory activities. On the other hand, doses of 50, 100 and 200mg/Kg oral water fraction showed their maximum anti-inflammatory activity of 32.16% at the 3rd hour, 39.41% and 46.67% at the 5th hour, respectively. Paw edema inhibition of high doses of water fractions of *Ocimum suave* found to be comparable to that of aspirin.

Table 4: Anti-inflammatory activities of butanol and water fractions of *Ocimum suave* in comparison to that of aspirin on carrageenan-induced mouse paw edema.

Group	Dose (mg/Kg)	Anti-inflammatory activity (%A)				
		1h	2h	3h	4h	5h
Normal saline		—	—	—	—	—
Aspirin	200	28.52	37.42	34.70	41.18	40.00
Butanol fraction	50	8.77	9.84	13.78	16.96	19.42
	100	9.81	21.38	27.43	28.47	28.42
	200	19.43	29.49	31.15	30.64	31.41
Water residue	50	19.00	20.77	32.16	26.78	31.70
	100	26.11	36.49	36.70	35.06	39.41
	200	32.23	37.63	38.90	45.11	46.67

4.2. Effects of *Ocimum suave* on Histamine-induced Paw Edema

The effects of different doses of butanol and water fractions of *Ocimum suave* on histamine-induced paw edema are presented in table 5 and 6. Mice treated with both fractions reduced edema induced by histamine in a dose dependent manner. The butanol fraction significantly decreased inflammation ($P<0.05$ at 4th hour and $P<0.01$ at 2nd and 3rd hours) at a dose level of 50mg/Kg. This fraction also showed significant reduction of edema volume ($P<0.05$ at 5th hour; $P<0.01$ at 2nd and 4th hours and $P<0.001$ at 3rd hour) at 100mg/Kg dose. Butanol fraction significantly reduced histamine-induced edema throughout the experiment ($P<0.01$ at 4th and 5th hours; $P<0.001$ at 1st, 2nd, 3rd hours) at 200mg/Kg as compared to the control.

On the other hand, all doses of the aqueous fraction significantly reduced histamine induced paw volume throughout the observation time. Aqueous fraction significantly reduced inflammation (*P<0.01* at 1st, 4th, 5th hours; *P<0.001* at 2nd and 3rd hours) at 50mg/Kg. The result also showed that 100mg/Kg water fraction significantly decreased histamine-induced inflammation (*P<0.01* at 4th and 5th hour; *P<0.001* at 1st, 2nd, 3rd hours). The highest dose, 200mg/Kg, reduced the increase in paw volume at the fifth hour (*P<0.01*) and at the first, second, third and fourth hours (*P<0.001*) as compared to the control.

The effect produced by 200mg/Kg butanol fraction and all doses of aqueous fraction were not significantly different from the standard drug throughout the experiment. However, the butanol fraction at 50 (at all hours except at 5th hour) and 100mg/Kg (at 1st hour) produced significantly lower effect than aspirin on histamine-induced paw edema (table 5).

Table 5: Effects of oral butanol and water fractions of *Ocimum suave* on histamine-induced mouse paw edema.

Group	Dose (mg/Kg)	Mean Inflammation (%I) ± S.E.M				
		1h	2h	3h	4h	5h
Normal saline		45.73±1.20	54.47±1.89	48.08±2.16	43.54±3.34	40.22±3.45
Aspirin	200	30.25 ± 2.07^{xa}	31.67 ± 2.66^{xa}	26.57 ± 2.25^{xa}	24.11 ± 2.49^{xb}	22.63 ± 2.65^{xb}
Butanol fraction	50	43.74 ± 1.79^{ya}	$39.95\pm2.57^{xb,\ yc}$	$36.08\pm2.17^{xb,\ yb}$	$32.80\pm2.58^{xc,\ yc}$	32.33±5.38
	100	40.54 ± 3.72^{yc}	38.06 ± 3.53^{xb}	32.35 ± 2.47^{xa}	30.03 ± 1.87^{xb}	29.32 ± 3.25^{xc}
	200	34.30 ± 1.91^{xa}	34.11 ± 3.24^{xa}	29.24 ± 2.93^{xa}	26.95 ± 3.18^{xb}	25.48 ± 2.05^{xb}
Water residue	50	35.38 ± 2.16^{xb}	34.09 ± 2.90^{xa}	32.94 ± 2.46^{xa}	29.54 ± 1.53^{xb}	26.34 ± 1.08^{xb}
	100	32.80 ± 2.21^{xa}	30.55 ± 2.37^{xa}	28.63 ± 2.06^{xa}	25.21 ± 1.44^{xb}	22.11 ± 1.70^{xb}
	200	28.98 ± 2.14^{xa}	28.68 ± 3.70^{xa}	24.15 ± 3.01^{xa}	22.42 ± 2.72^{xa}	22.02 ± 2.67^{xb}

^{x}: relative to control, ^{y}: relative to standard: $^{a}P< 0.001$, $^{b}P< 0.01$, $^{c}P<0.05$.

The anti-inflammatory activities of the fractions of *Ocimum suave* are shown in table 6. Doses of 100 and 200mg/Kg of the water fraction exhibited greater inhibition of paw edema than the butanol fraction. The paw edema inhibition of the highest dose, 200mg/Kg, of butanol fraction is comparable to the smallest dose, 50mg/Kg, of aqueous fraction. At a dose of 200mg/Kg peak

anti-inflammatory activities of 39.18% for butanol fraction and 49.77% for water fraction were observed at the 3rd hour. The anti-inflammatory activities of high doses of water fractions of *Ocimum suave* were found to be comparable to that of the standard, aspirin (table 6).

Table 6: Anti-inflammatory activities of butanol and water fractions of *Ocimum suave* in comparison to that of aspirin on histamine-induced mouse paw edema.

Group	Dose (mg/Kg)	Anti-inflammatory activity (%A)				
		1h	2h	3h	4h	5h
Normal saline		—	—	—	—	—
Aspirin	200	33.85	41.86	44.74	44.63	43.73
Butanol fraction	50	4.53	26.66	24.96	24.67	19.62
	100	11.35	30.13	32.72	31.03	27.10
	200	24.99	37.38	39.18	38.10	36.65
Water residue	50	22.63	37.42	31.49	32.15	34.51
	100	28.27	43.91	40.45	42.10	45.03
	200	36.63	47.35	49.77	48.51	45.25

4.3. Effects of *Ocimum suave* on Serotonin-induced Paw Edema

Table 7 and 8 shows the time course of anti-inflammatory effects of fractions of the aqueous extract after sub-plantar injection of serotonin. Both the butanol and aqueous fractions of *Ocimum suave* decreased serotonin-induced paw edema in a dose dependent manner. Both fractions significantly reduced paw volume at all doses. The butanol fraction at 50mg/Kg ($P<0.05$ at 5th hour), 100mg/Kg ($P<0.05$ at 3rd, 4th and 5th hours) and 200mg/Kg ($P<0.05$) significantly decreased inflammation induced by serotonin as compared to vehicle treated group.

Table 7 also showed that aqueous fraction significantly reduced serotonin-induced paw volume throughout the observation time. At a dose of 50mg/Kg, aqueous fraction significantly decreased paw edema ($P<0.05$). It was also observed that 100mg/Kg and 200mg/Kg of the aqueous fraction produced a significant reduction of inflammation ($P<0.01$). The anti-

inflammatory activities of high doses of water residue were comparable to that of the standard drug, aspirin (table 8).

The result also showed that the butanol fraction at 50 (at 1st and 2nd hours) and 100mg/Kg (at 1st hour) produced significantly lower anti-inflammatory effect than aspirin on serotonin-induced paw edema. The effect produced by 200mg/Kg butanol fraction and all doses of aqueous fraction were not, however, significantly different from the standard drug, aspirin throughout the observation time (table 7).

Table 7: Effects of oral butanol and water fractions of *Ocimum suave* on serotonin-induced mouse paw edema.

Group	Dose (mg/Kg)	Mean Inflammation (%I) ± S.E.M				
		1h	2h	3h	4h	5h
Normal saline		36.41±2.55	41.29±3.12	48.31±3.24	41.36±3.71	37.32±2.98
Aspirin	200	25.78±1.16^{xb}	28.54±1.62^{xb}	31.69±2.70^{xb}	26.57±2.29^{xb}	24.20±2.39^{xb}
Butanol fraction	50	34.80±3.15^{yc}	37.27±2.53^{yc}	41.28±4.09	33.86±4.75	29.32±1.79^{xc}
	100	32.53±2.28^{yc}	34.75±2.38	37.48±2.56^{xc}	31.37±1.44^{xc}	28.64±2.19^{xc}
	200	28.04±2.43^{xc}	30.79±2.27^{xc}	36.75±2.47^{xc}	30.54±2.22^{xc}	27.27±1.87^{xc}
Water residue	50	29.09±2.00^{xc}	31.22±1.13^{xc}	36.85±1.58^{xc}	30.57±1.18^{xc}	27.02±2.13^{xc}
	100	25.90±0.58^{xb}	28.55±1.64^{xb}	31.66±2.99^{xb}	26.70±1.18^{xb}	24.82±1.85^{xb}
	200	24.97±1.42^{xb}	26.73±1.63^{xb}	30.17±1.74^{xb}	24.55±1.67^{xb}	22.62±1.88^{xb}

^{x}: relative to control, ^{y}: relative to standard: $^{a}P< 0.001$, $^{b}P< 0.01$, $^{c}P<0.05$.

Higher anti-inflammatory activity was exhibited by the aqueous fraction at 100mg/Kg and 200mg/Kg dose levels as compared to that of the butanol fraction (table 8). The butanol and aqueous fractions at a dose of 200mg/Kg showed 26.93% at the 5th hour, and 40.64% at the 4th hour as their maximum anti-inflammatory activity, respectively (table 8).

Table 8: Anti-inflammatory activities of butanol and water fractions of *Ocimum suave* in comparison to that of aspirin on serotonin-induced mouse paw edema.

Group	Dose (mg/Kg)	Anti-inflammatory activity (%A)				
		1h	2h	3h	4h	5h
Normal saline		—	—	—	—	—
Aspirin	200	29.19	30.88	34.40	35.76	35.16
Butanol fraction	50	4.42	9.74	14.55	18.13	21.44
	100	10.66	15.84	22.42	24.15	23.26
	200	22.99	25.43	23.93	26.16	26.93
Water residue	50	20.10	24.39	23.72	26.09	27.59
	100	28.87	30.85	34.46	35.44	33.49
	200	31.42	35.26	37.55	40.64	39.39

5. DISCUSSION

In the present study, anti-inflammatory effects of ethanol and aqueous extract, and fractions of the most active extract i.e. aqueous extract of *Ocimum suave* were investigated after sub-plantar injection of carrageenan, histamine and serotonin in mouse paw.

This study showed that the crude extracts (ethanol and aqueous) and fractions (butanol and water) of *Ocimum suave* possess anti-inflammatory activities in mice model. The observation that the aqueous extract of *Ocimum suave* exhibited greater anti-inflammatory activity than the ethanol extract, more of the active principle (s) responsible for the anti-inflammatory activity might be present in higher concentration in the aqueous extract. In addition, greater paw edema inhibition of the water residue of the aqueous extract indicates that the active principle (s) might be more soluble in water than butanol.

The presence of edema is one of the prime signs of inflammation (Yonathan et al., 2006). It has been documented that carrageenan induced rat paw edema is suitable *in vivo* model to study anti-inflammatory drugs both steroidal and non-steroidal since it involves several mediators (Mequanint et al., 2011). This method was chosen for the present study since edema induced by carrageenan is the most prominent acute experimental model in search for new anti-inflammatory drugs (Yonathan et al., 2006). In addition, it is a method that has been frequently used to assess the anti-edematous effect of natural products (Panthong et al., 2007). Furthermore, carrageenan is devoid of apparent systemic effects and exhibits a high degree of reproducibility (Kale et al., 2007).

The local injection of carrageenan induces inflammatory reactions in two different phases (Carey et al., 2010). The initial phase, which occurs between 0 and 2.5 h after the injection of the phlogistic agent, has been attributed to the action of mediators such as histamine, 5-HT and BK on vascular permeability (Yonathan et al., 2006). It has been reported that histamine and 5-HT are mainly released during the first 1.5 h while BK is released within 2.5 h after carrageenan injection (Panthong et al., 2007). The late phase is a result of overproduction of PGs in tissues and may occur from 2.5 to 6 h post-carrageenan injection (Mequanint et al., 2011). This late phase appears to be the most interesting phase in terms of inflammatory

processes. Thus, the maximal vascular response as determined with leukocyte migration to the inflamed area, also reaches its maximum level in this phase. It is well established that PGs, by virtue of their activity as modulators of inflammatory responses, have a major role in the inflammatory mechanism (Panthong et al., 2007).

The carrageenan-induced hind paw edema in rat is known to be sensitive to COX inhibitors, but not to LOX inhibitors, and has been used to evaluate the effect of NSAIDs which primarily inhibit the COX involved in PGs synthesis. It has been demonstrated that the suppression of carrageenan-induced hind paw edema after the third hour correlates reasonably with therapeutic doses of most clinically effective anti-inflammatory agents (Panthong et al., 2007).

Based on the results of this study, both ethanol and aqueous extract and fractions (butanol and water) of aqueous extract of *Ocimum suave* were able to effectively inhibit the increase in paw volume during both phases of carrageenan-induced inflammation. This indicates that the active component (s) of the plant may suppress both early and late phases of inflammation perhaps by inhibiting the release and/or activity of the inflammatory mediators involved in carrageenan-induced paw edema. The anti-inflammatory activities of the fractions of the aqueous extract might be associated to their anti-histamine or anti 5-HT activities observed in this study as these fractions also suppressed histamine and 5-HT induced inflammation. The strong anti-inflammatory activity of *Ocimum suave*, thus, might be by interfering with histamine, 5-HT and PGs synthesis or activity.

Previous phytochemical investigation of ethanol and aqueous extracts has revealed the presence of phenolic acids, flavonoids, glycosides, sugars (Makonnen et al., 2003b; Tan et al., 2008) and triterpenes (Tan et al., 2008) as major chemical constituents. Flavonoids isolated from different medicinal plants have been shown to possess anti-oxidant and anti-inflammatory activities (Nijveldt et al., 2001; Havsteen, 2002) and the anti-inflammatory property of triterpenes was also reported (Gautam and Jachak, 2009). These different compounds, thus, may be responsible for the anti-inflammatory activity of *Ocimum suave*.

Both extracts and fractions of *Ocimum suave* showed persistent anti-inflammatory activity during the five hours observation time. This study may give hint about the onset and duration

of action of the extracts and fractions of the plant investigated. Though it is difficult to speculate the time of onset for anti-inflammatory activity, both aqueous and ethanol extracts of the leaf of *Ocimum suave* for analgesic activity showed rapid onset, i.e. starting from ten minutes (Makonnen et al., 2003a). The duration, however, seems to be fairly long. To get full pictures of their pharmacokinetic profiles, further investigation should be undertaken.

6. CONCLUSION AND RECOMMENDATION

In conclusion, this study has shown that ethanol and aqueous extract of *Ocimum suave* does possess significant anti-inflammatory activities with high doses of the aqueous extract being more active. In addition, the butanol and water fractions of the aqueous extract of *Ocimum suave* have been confirmed to exert anti-inflammatory activity in which the water residue produced higher anti-inflammatory activity. The results, thus, might support the traditional use of this plant in inflammatory process. Further investigation, however, should be pursued after isolation and characterization of the active principle (s) in order to come up with the active compound (s) responsible for the anti-inflammatory properties of the plant. Moreover, other studies should be performed to confirm the exact mechanism (s) and anti-inflammatory activity of the plant in chronic inflammatory models.

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